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ORIGINAL INVESTIGATION

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Adenosinergic modulation of the discriminative-stimulus effects of methamphetamine in rats

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Abstract *Rationale:* A_1 and A_{2A} adenosine receptors are co-localized with dopamine D_1 and D_2 receptors, respectively, and their stimulation attenuates dopaminergic functioning. *Objective:* To test whether adenosine antagonists with different selectivities for A_1 and A_{2A} receptors mimic the discriminative-stimulus effects of dopamine releaser methamphetamine. *Methods:* Effects of the A_1 antagonist DPCPX, the preferential A_{2A} antagonist DMPX and the non-selective adenosine antagonist caffeine were evaluated in Sprague-Dawley rats trained to discriminate 1.0 mg/kg, IP, methamphetamine from saline under a fixed-ratio 10 schedule of food presentation. *Results:* The A_1 antagonist DPCPX (1.0–10.0 mg/kg) failed to substitute for methamphetamine. However, 5.6 mg/kg DPCPX shifted the methamphetamine dose-response curve to the left. The A_{2A} antagonist DMPX (1.8–18.0 mg/kg) produced about 70% methamphetamine-appropriate responding and the non-selective antagonist caffeine (3.0–56.0 mg/kg) about 50% methamphetamine-appropriate responding at the highest tested doses. Both DMPX (5.6 mg/kg) and caffeine (30.0 mg/kg) shifted the methamphetamine dose-response curve to the left. Methamphetamine-like effects of DMPX were blocked fully by the D_2 antagonist spiperone (0.18 mg/kg) and partially by the D_1 antagonist SCH-23390 (0.018 mg/kg). *Conclusions:* Antagonism at A_{2A} adenosine receptors directly mimics the discriminative-stimulus effects of methamphetamine through the interaction with dopamine receptors. Antagonism at A_1 adenosine receptors potentiates effects of lower methamphetamine doses and thus plays a rather indirect, modulatory role.

Keywords Methamphetamine · Adenosine · DPCPX · DMPX · Caffeine · SCH 23390 · Spiperone · Drug discrimination · Rat

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Introduction

Methamphetamine's neurochemical actions appear to be mediated by release of dopamine, norepinephrine and serotonin from nerve terminals (e.g. Kuczenski et al. 1995). Although the involvement of adrenergic and serotonergic systems in the discriminative-stimulus and other behavioral actions of methamphetamine has been reported (Sasaki et al. 1995; Munzar and Goldberg 1999; Munzar et al. 1999a, 1999b, 2002), activation of dopamine neurons by methamphetamine seems to play a more important role in its discriminative-stimulus effects and appears to represent a final common pathway responsible for socalled modulatory effects of other neurotransmitter systems (Munzar and Goldberg 2000). It has furthermore been shown that both dopamine D_1 and D_2 receptor agonists are able, at least partially, to mimic subjective effects of methamphetamine under the drug discrimination paradigm (Tidey and Bergman 1998; Munzar and Goldberg 2000).

The interaction between dopaminergic and adenosine receptors has been extensively studied and an antagonistic influence of endogenous adenosine on dopaminergic functions has been demonstrated (Ferré et al. 1997). In the brain, endogenous adenosine acts primarily by stimulation of two adenosine receptors, A_1 and A_{2A} (e.g. Fredholm et al. 2000; Klotz 2000). A_1 adenosine receptors and dopamine D_1 receptors form functional heteromeric complexes (Ginés et al. 2000) and stimulation of A_1 adenosine receptors decreases both neurochemical and behavioral effects of D_1 receptor stimulation (e.g. Ferré et al. 1994, 1999). Similarly, A_{2A} adenosine receptors are co-localized with D_2 receptors and their stimulation

counteracts effects of D_2 receptor stimulation (Ferré et al. 1991, 1997). This would predict that behavioral effects of methamphetamine and of other psychomotor stimulants with a similar mechanism of action are modulated by compounds acting at both A_1 and A_{2A} adenosine receptors.

Indeed, it has been demonstrated that both A_1 and A_{2A} adenosine receptors play a role in the rewarding effects of cocaine and that their activity is altered during cocaine withdrawal (Manzoni et al. 1998; Kuzmin et al. 1999; Baldo et al. 1999; Fiorillo and Williams 2000; Knapp et al. 2001). Adenosine A_1 and A_{2A} receptors also play a role in amphetamine-induced locomotion (Turgeon et al. 1996; Gasior et al. 2000) and stereotypy (Poleszak and Malec 2000) and in expression of methamphetamine-induced sensitization to locomotor activity (Shimazoe et al. 2000) and in methamphetamine-induced dopamine release in rat striatum (Golembiowska and Zylewska 1998). The importance of adenosine in the modulation of actions of amphetamine is supported by recent findings of decreased behavioral effects of amphetamine in mice lacking A_{2A} adenosine receptors (Chen et al. 2000), probably reflecting complex neuroadaptation processes in these transgenic animals. Finally, the nonselective adenosine antagonist caffeine potentiates the behavioral responses to amphetamine and cocaine in rats responding for food under a fixed-interval schedule of food reinforcement (Jaszyna et al. 1998). Caffeine also has been found to potentiate the discriminative-stimulus effects of amphetamine and cocaine and to mimic partially their discriminative-stimulus effects upon substitution (Schechter 1977; Gauvin et al. 1990; Young et al. 1998; reviewed by Garret and Griffiths 1997). However, the role of A_1 and A_{2A} adenosine receptors in the discriminative-stimulus effects of either cocaine or amphetamines is still unknown.

The aim of the present study was to assess the relative role of A_1 and A_{2A} adenosine receptors in modulating the discriminative-stimulus effects of methamphetamine by testing several adenosine antagonists in rats discriminating methamphetamine from saline. Adenosine antagonists studied included DPCPX, an A_1 adenosine antagonist (Lohse et al. 1987; Klotz 2000), DMPX, a preferential A_{2A} adenosine antagonist (Seale et al. 1988; Muller et al. 1997) and the nonselective adenosine antagonist caffeine (Fredholm et al. 1999).

Materials and methods

Subjects

Sixteen male Sprague-Dawley rats (Charles River, Wilmington, Mass., USA) experimentally naive at the start of the study and initially weighing 280–350 g were housed individually. Their body weights were gradually reduced to approximately 80% of free feeding by limiting daily access to food. Water was available ad libitum. All rats were housed in a temperature- and humidity-controlled room and were maintained on a 12-h light/dark cycle; the lights were on from 6:45 a.m. to 6:45 p.m. Experiments were conducted during the light phase.

Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse, NIH, and the Guide for Care and Use of Laboratory Animals (National Research Council 1996).

Apparatus

Twelve standard operant conditioning chambers (Coulbourn Instruments, Lehigh Valley, Pa., USA) were used. Each chamber contained two levers, separated by a recessed tray into which a pellet dispenser could deliver 45 mg food pellets (F0021; Bioserv, Frenchtown, N.J., USA). Each press of a lever with force of 0.4 N through 1 mm was recorded as a response and was accompanied by an audible click. The operant conditioning chambers were controlled by microcomputers using the MED Associates MED-PC software package (Med Associates Inc., East Fairfield, Vt., USA).

Drug-discrimination procedure

Rats were trained as described previously (Yasar et al. 1993; Munzar and Goldberg 1999, 2000; Munzar et al. 1999a, 1999b) under a discrete-trial schedule of food-pellet delivery to respond on one lever after an injection of a training dose of 1.0 mg/kg methamphetamine and on the other lever after an injection of 1.0 ml/kg saline vehicle. Injections of methamphetamine or saline were given IP 15 min before the start of the session. At the start of the session, a white house light was turned on and in its presence the rats were required to make ten consecutive responses (fixed-ratio 10 schedule of food delivery; FR10) on the lever appropriate to the pre-session treatment. The completion of ten consecutive responses on the correct lever produced delivery of a 45 mg food pellet and initiated a 45-s time-out during which lever-press responses had no programmed consequences and the chamber was dark. Responses on the incorrect lever had no programmed consequences other than to reset the FR requirement on the correct lever. After each time-out, the white house light was again turned on and the next trial began. Each session ended after completion of 20 fixed-ratio trials or after 30 min elapsed, whichever occurred first.

Discrimination-training sessions were conducted 5 days per week under a double alternation schedule (i.e. DDSSDDSS etc., D=drug, methamphetamine; S=saline). Training continued until there were eight consecutive sessions during which rats completed at least 90% of their responses during the session on the correct lever and no more than four responses occurred on the incorrect lever during the first trial. Test sessions with other doses and other drugs were then initiated.

During the test sessions, different doses of three adenosine antagonists were administered either alone or together with different methamphetamine doses or with dopaminergic antagonists. Test sessions were identical to training sessions with the exception that ten consecutive responses on either one of the two levers ended the trial. Switching responding from one lever to the other lever reset the ratio requirement. In a test phase, a single alternation schedule was introduced and test sessions were usually conducted on Tuesdays and Fridays. Thus, a 2-week sequence starting on Monday was: DTSDTSTDST (T=test). In this way, test sessions occurred with equal probability after saline and drug sessions. Test sessions were conducted only if the criterion of 90% accuracy and not more than four incorrect responses during the first trial was maintained in the two preceding training sessions.

Drugs

S(+)-Methylamphetamine HCl (methamphetamine), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 3,7-dimethyl-1-propargylxanthine (DMPX; PD 116,948), caffeine, spiperone HCl and R(+)-SCH-23390 HCl were purchased from RBI (Research Biochemicals

International, Natick, Mass., USA). Doses of methamphetamine, spiperone, and SCH-23390 refer to the weight of the salt whereas doses of DPCPX, DMPX, and caffeine refer to the weight of the drug. Methamphetamine, caffeine, spiperone and SCH-23390 were dissolved in 0.9% NaCl. DPCPX and DMPX were dissolved in 3% polyoxyethylene sorbitan mono-oleate (Tween 80; Sigma, St Louis, Mo., USA). All compounds were slightly heated and/or sonicated as needed. Most drugs were injected in a volume of 1.0 ml/kg, the highest tested doses of caffeine (56.0 mg/kg), DPCPX (10.0 mg/kg) and DMPX (10.0 and 18.0 mg/kg) were injected in a volume of 2.0 ml/kg due to solubility constraints. Methamphetamine, caffeine, DPCPX and DMPX were administered IP. Spiperone and SCH-23390 were administered SC. Methamphetamine and adenosine antagonists (caffeine, DPCPX, DMPX) were injected 15 min before the session whereas spiperone and SCH-23390 were injected 25 min before the session.

A range of doses of each drug was tested in the drug-discrimination study and dose was increased until there was either complete generalization to the methamphetamine-training stimulus or until the test drug produced a marked and significant decrease in response rates. Effects of adenosine antagonists alone were usually tested first. Effects of selected doses of each antagonist and of its vehicle on the methamphetamine dose-response curve were then established.

After that, effects of dopaminergic antagonists and of their vehicles on the discriminative-stimulus effects of the training dose of methamphetamine and on the generalization to the methamphetamine-training stimulus produced by selected doses of DMPX and caffeine were evaluated. Only rats in which these selected doses of caffeine and DMPX produced at least partial substitution for the 1.0 mg/kg training dose of methamphetamine during initial tests were included in this part of the study. Rats that showed no generalization to the methamphetamine-training stimulus after substitution of DMPX or caffeine were excluded from these tests, since some level of generalization had to be present for antagonism studies to be meaningful. Two dopaminergic antagonists (D_2 antagonist spiperone and D_1 antagonist SCH 23390) were used in this assay. We previously reported that both antagonists dose dependently and completely antagonized methamphetamine's discriminative-stimulus effects (dose ranges tested were 0.003–0.056 mg/kg for SCH 23390 and 0.01–0.3 mg/kg for spiperone; Munzar and Goldberg 2000). Doses of dopaminergic antagonists (0.18 mg/kg spiperone and 0.018 mg/kg SCH-23390) used in the present study were selected as the doses that produced approximately equal, partial blockades of methamphetamine's discriminative-stimulus effects in our previous study (Munzar and Goldberg 2000). Thus, the aim of the present study was to compare relative D_1 and D_2 involvement in the effects of adenosinergic antagonists and methamphetamine. Not all the compounds were tested in all the subjects. Generally, DPCPX and DMPX were tested in the same subjects whereas approximately half of the subjects used for testing caffeine effects were not tested with two other adenosine antagonists.

Data analysis

Discriminative-stimulus data were expressed as the percentage of the total responses on both levers that were made on the methamphetamine-appropriate lever. Complete generalization to the methamphetamine training dose was defined as 80% or more of responses on the methamphetamine-appropriate lever, with no generalization defined as 25% or less of responses on the methamphetamine-appropriate lever. Response-rate data were expressed as responses per second averaged over the session, with responding during time-out periods not included in calculations. The data from sessions during which rats did not complete at least one fixed-ratio were excluded from analysis of drug-lever selection. All results are presented as group means (±SEM).

Statistical analysis in substitution tests and in tests analyzing effects of dopaminergic antagonists on drug-lever selection induced by adenosinergic antagonists was done by using one-way ANOVA for repeated measures. Significant main effects were analyzed further by subsequent paired comparisons with vehicle control (responding after vehicle injections in substitution tests or after vehicle pretreatment in antagonism tests) using post-hoc Dunnett's test. Shifts in the dose-response curves were statistically evaluated by two-way ANOVA for repeated measures. In addition, theoretically additive values of drug combinations were calculated and compared with experimental values actually obtained in order to find out whether drug combinations produced simple additive or more than additive effects. Theoretically additive values were individually calculated for each rat as described previously (Munzar et al. 2002) by adding the effect of each pretreatment drug when administered alone to the effects of each dose of methamphetamine when administered together with the vehicle. Since 100% was the maximal achievable value, all sums greater than 100% were adjusted to this value. Changes were considered to be significant when $P<0.05$. SigmaStat program (Jandel Scientific, USA) was used.

Results

Rats started to discriminate methamphetamine from saline reliably after approximately 30 days of training, but reaching the final level of accuracy (eight consecutive sessions with at least 90% of the responses on the correct lever and no more than four incorrect responses during the first trial) required 35–85 sessions of training. Rates of responding during the training sessions were stable across sessions during the study and were slightly lower after methamphetamine than after saline pretreatment, as in previous studies using the same 1.0 mg/kg training dose of methamphetamine (Munzar and Goldberg 1999, 2000; Munzar et al. 1999a, 1999b).

Figure 1 shows stimulus-generalization results and rates of responding obtained during sessions when methamphetamine and three adenosine antagonists were tested for their ability to substitute for the 1.0 mg/kg training dose of methamphetamine. Figure 2 shows the effects of a selected dose of each of three adenosine antagonists on the methamphetamine dose-response curve. Figure 3 shows effects of the dopamine D_2 antagonist spiperone and the dopamine D_1 antagonist SCH23390 on the discriminative-stimulus effects of the 1.0 mg/kg training dose of methamphetamine, 10.0 mg/kg dose of DMPX and 30.0 mg/kg dose of caffeine.

When methamphetamine dose was varied, there was a dose-dependent generalization to the 1.0 mg/kg methamphetamine training stimulus [one-way ANOVA for repeated measures; $F(4,60)=45.596$, $P<0.001$, Fig. 1]. In contrast, the selective A_1 adenosine antagonist DPCPX failed to generalize significantly to the 1.0 mg/kg methamphetamine training stimulus (Fig. 1) at any dose tested (1.0–10.0 mg/kg). The 5.6 and 10.0 mg/kg doses of DPCPX significantly attenuated rates of responding [one-way ANOVA for repeated measures; $F(5,55)=3.252$, $P=0.012$] and 10.0 mg/kg DPCPX produced a complete blockade of responding in one of 12 rats. For this reason, the lower 5.6 mg/kg dose of DPCPX was selected for testing in combination with methamphetamine. This dose of DPCPX (5.6 mg/kg) produced a shift to the left of the methamphetamine dose-response curve (Fig. 2), which was significant as revealed by two-way ANOVA

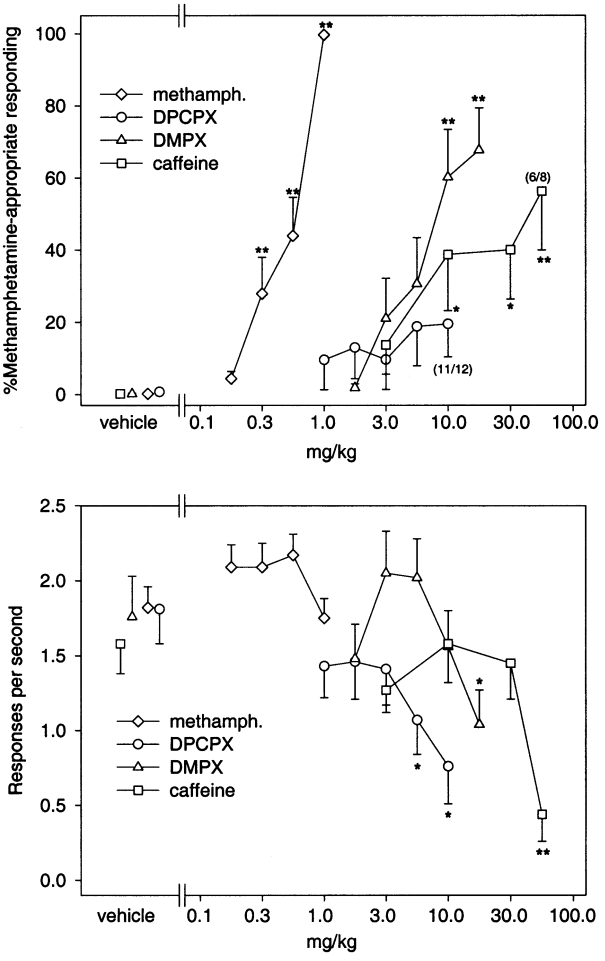

Fig. 1 Effects of IP pretreatment with methamphetamine (*diamonds*), DPCPX (*circles*), DMPX (*triangles*) and caffeine (*squares*) in rats trained to discriminate 1.0 mg/kg IP of methamphetamine from saline. Data are means (±SEM) from 16 (methamphetamine), 12 (DPCPX), ten (DMPX) or eight (caffeine) rats. The percentage of methamphetamine-appropriate responding is shown as a function of dose during substitution test sessions (*upper panel*). Response rates are expressed as responses per second (*lower panel*). **P*<0.05, ***P*<0.01, post-hoc comparison with the vehicle pretreatment after significant ANOVA for repeated measures main effect, Dunnett's test. *Numbers in parentheses* at higher doses indicate the number of rats that completed at least one fixed-ratio during the session relative to the total number of rats tested

for repeated measures [$F(1,22)=11.187$, $P=0.007$]. The effect of the DPCPX-methamphetamine combination was clearly more than additive, since there was a statistically significant difference between curves actually obtained and the calculated additive curves [$F(1,11)=6.956$, $P=0.023$].

The preferential A_{2A} adenosine antagonist, DMPX, produced a partial but statistically significant generalization to the methamphetamine training stimulus at doses of 10.0 and 18.0 mg/kg [one-way ANOVA for repeated measures; $F(5,45)=11.983$, $P<0.001$; Fig. 1]. The level of methamphetamine-appropriate responding at the 10.0 mg/kg dose of DMPX was about 60%. Increasing the dose of DMPX to 18.0 mg/kg only increased drug-lever selection by about 10%, but it significantly decreased rates of responding [one-way ANOVA for repeated measures; $F(5,45)=4.48$, $P=0.002$]. Higher doses could not be tested due to solubility constraints. When a dose of 5.6 mg/kg DMPX, which did not produce significant generalization to the methamphetamine training stimulus and did not significantly change response rates when given alone, was administered with different doses of methamphetamine there was a marked leftward and upward shift in the methamphetamine dose-response curve (Fig. 3), which was significant as revealed by two-way ANOVA for repeated measures [$F(1,14)=15.096$, $P=0.006$]. Although the effects of DMPX-methamphetamine combinations appeared to be more than additive (see grey symbols in Fig. 3, middle panel), there was no statistically significant difference between the curve actually obtained and the calculated additive curve [$F(1,14)=2.306$, $P=0.173$).

The non-selective adenosine antagonist caffeine produced a partial but statistically significant generalization to the methamphetamine training stimulus at the doses of 10.0–56.0 mg/kg (Fig. 1) with a maximum of about 50% methamphetamine-appropriate responding [one-way ANOVA for repeated measures; $F(4,26)=4.895$, $P=0.004$]. After 56.0 mg/kg caffeine, there was a significant [one-way ANOVA for repeated measures; $F(4,28)=7.087$, $P<0.001$] decrease in rates of responding and two of eight subjects did not complete even a single fixed-ratio. When a dose of 30.0 mg/kg caffeine, which produced levels of methamphetamine-appropriate responding comparable to these produced by the 5.6 mg/kg dose of DMPX, used in combination experiments (see above), was coadministered with different doses of methamphetamine, there was a marked upward shift in the methamphetamine dose-response curve (Fig. 3). This upward shift in the methamphetamine dose-response curve was significant, as revealed by two-way ANOVA for repeated measures [$F(1,16)=28.138$, $P<0.001$]. The effect of caffeine-methamphetamine combinations were more than additive, since there was a statistically significant difference between the curve actually obtained and the calculated additive curve [$F(1,8)=7.724$, $P=0.024$]. Rates of responding were, however, markedly reduced by this combination of treatments.

Subsequently, effects of two dopaminergic antagonists and their vehicles on the discriminative-stimulus effects of the 1.0 mg/kg training dose of methamphetamine and of doses of 10.0 mg/kg DMPX and 30.0 mg/kg caffeine, which partially generalized to the 1.0 mg/kg methamphetamine training stimulus, were evaluated. A dose of 0.18 mg/kg spiperone, a D_2 dopamine antagonist, partially but significantly attenuated methamphetamine's discriminative-stimulus effects when coadministered with the training dose of methamphetamine (Fig. 3); methamphetamine-appropriate responding decreased to about 60% [one-way ANOVA for repeated measures; $F(1,6)=6.868$, $P=0.04$], as reported previously (Munzar and Goldberg 2000). When the same dose of spiperone (0.18 mg/kg) was coadministered with 10.0 mg/kg DMPX there was a marked reduction in methamphet-

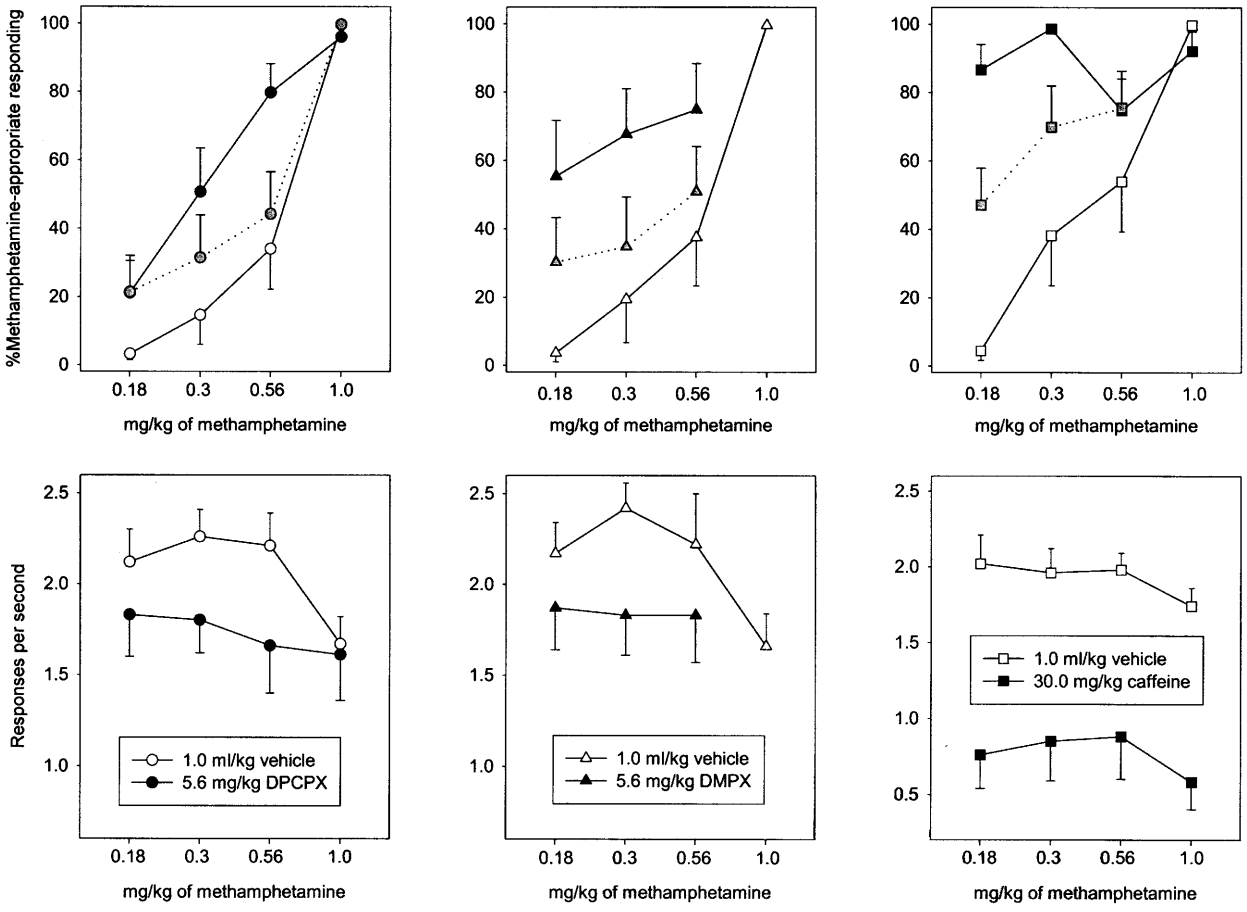

Fig. 2 Methamphetamine dose-response curves after IP pretreatments with 1.0 ml/kg vehicle (*open symbols*), 5.6 mg/kg DPCPX (*filled circles*), 5.6 mg/kg DMPX (*filled triangles*) and 30.0 mg/kg caffeine (*filled squares*). *Grey symbols* connected with dotted lines represent calculated curves, which show theoretical additive effects of adenosinergic antagonists and methamphetamine. Data are means (±SEM) from eight (DMPX), nine (caffeine) or 12 (DPCPX) rats. The percentage of methamphetamine-appropriate responding is shown as a function of dose of methamphetamine (*upper panels*). Response rates are expressed as responses per second (*lower panels*)

amine-appropriate responding from about 95% to about 25% drug-lever selection [one-way ANOVA for repeated measures: $F(1,5)=35.576$, $P=0.002$; Fig. 3]. Thus, spiperone blocked generalization of DMPX to the methamphetamine-training stimulus. Similarly, spiperone appeared to attenuate the discriminative-stimulus effects of 30.0 mg/kg of caffeine, but this effect did not reach significance ($P>0.05$). Coadministration of spiperone with methamphetamine, DMPX or caffeine resulted in pronounced decreases in rates of responding but all the subjects completed at least three fixed-ratio trials (Fig. 3, lower panels).

Like spiperone, a dose of 0.18 mg/kg SCH 23390 partially, but significantly, attenuated methamphetamine's discriminative-stimulus effects when coadministered with the training dose of methamphetamine (Fig. 3); methamphetamine-appropriate responding decreased to about 60% [one-way ANOVA for repeated measures: $F(1,7)=15.228$, P=0.006] with only modest decreases in rates of responding, as reported previously (Munzar and Goldberg 2000). SCH 23390 also partially, but significantly, blocked methamphetamine-like responding induced by DMPX 10.0 mg/kg [methamphetamine-appropriate responding decreased to about 32%; one-way ANOVA for repeated measures: $F(1,3)=11.145$, $P=0.044$], but this finding was compromised by the failure to emit a single response in two of six rats tested (Fig. 3). When SCH 23390 was coadministered with 30.0 mg/kg caffeine, responding was completely eliminated in five of nine subjects, which precluded evaluation of the effects of this combination on drug-lever selection (data not shown).

Discussion

In the present study, effects of three adenosinergic antagonists were investigated in rats trained to discriminate injections of methamphetamine from injections of saline. Even though DPCPX, a selective A_1 adenosine antagonist, did not produce methamphetamine-like discriminative-stimulus effects upon substitution, it shifted the methamphetamine dose-response curve to the left. This would suggest that A_1 receptors are not directly involved in methamphetamine's discriminative-stimulus actions but their blockade can potentiate these actions of methamphetamine. Thus, A_1 receptors appear to play a rather

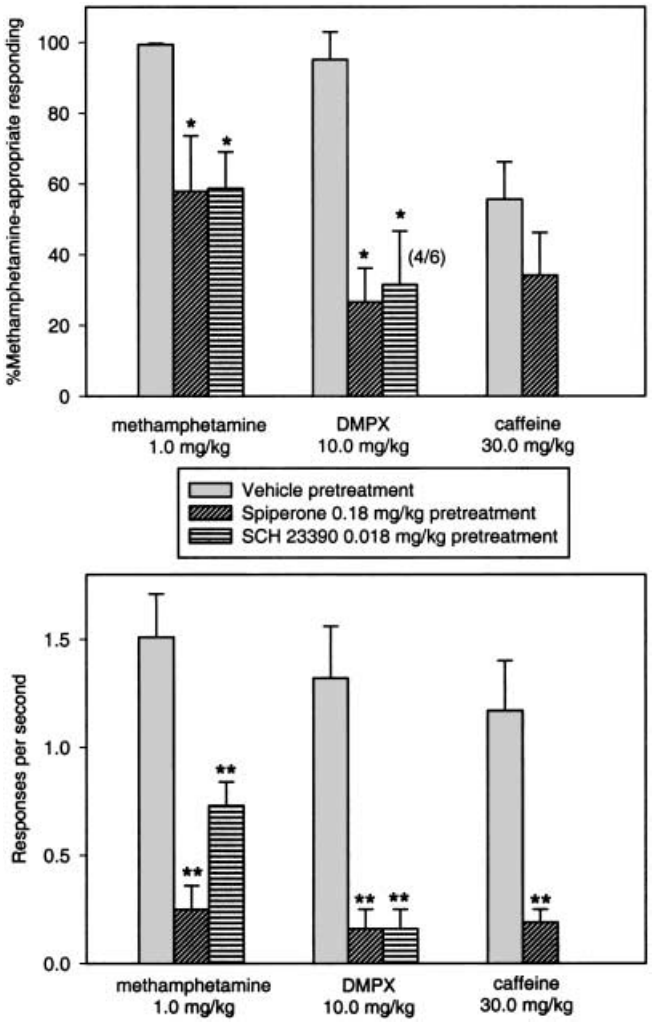

Fig. 3 Effects of SC pretreatment with vehicle (*left bars*), 0.18 mg/kg spiperone (*middle bars*) or with 0.018 mg/kg SCH 23390 (*right bars*) on methamphetamine (1.0 mg/kg IP, *left panels*), DMPX (10.0 mg/kg IP, *middle panels*), and caffeine (30.0 mg/kg IP, *right panels*) substitution for methamphetamine. The percentage of methamphetamine-appropriate responding is shown in the *upper panels*. Response rates are expressed as responses per second (*lower panels*). Data are means (±SEM) from seven or eight (methamphetamine and caffeine) or six (DMPX) rats. The effects of DMPX-spiperone, DMPX-SCH 23390 and caffeine-spiperone combinations were tested in a subgroup of rats that showed at least partial generalization to the methamphetamine-training stimulus during initial tests with DMPX and caffeine. After caffeine-SCH 23390 combination, responding was completely eliminated in most subjects, which precluded evaluation of drug-lever selection. *Numbers in parentheses* indicate the number of rats completing at least one fixed-ratio trial during the session relative to the total number of rats tested. **P*<0.05, ***P*<0.01, post-hoc comparison with the vehicle pretreatment after significant ANOVA for repeated measures main effect, Dunnett's test

indirect, albeit important, modulatory role. In contrast to the A_1 antagonist DPCPX, the preferential A_{2A} antagonist DMPX produced almost complete generalization to the methamphetamine-training stimulus. This suggests that A_{2A} receptors play a more direct role in mediating the discriminative-stimulus effects of methamphetamine than A_1 receptors. This finding is consistent with numerous studies demonstrating a clearly stronger role of A_{2A} receptors than A_1 receptors in the behavioral and neurochemical effects of psychomotor stimulants. For example, A_{2A} agonists but not A_1 agonists prevented development of methamphetamine-induced sensitization to locomotor stimulant effects (Shimazoe et al. 2000). Also, pretreatment with an A_{2A} agonist but not with an A_1 agonist attenuated c-*fos* induction in the caudate-putamen and nucleus accumbens by amphetamine (Turgeon et al. 1996) and DMPX but not the A_1 antagonist CPT increased stereotypy induced by amphetamine (Poleszak and Malec 2000). Furthermore, locomotor stimulating effects of amphetamine were decreased in A_{2A} receptor deficient mice (Chen et al. 2000). Although A_{2A} receptors appear to play a more direct role than A_1 receptors in the motor effects of amphetamine, both receptors may play an equal role in processes of neuroadaptation to long term psychomotor stimulant exposure or during withdrawal from psychomotor stimulants, where the role of both A_{2A} (Baldo et al. 1999) and A_1 (Manzoni et al. 1998; Kuzmin et al. 1999; Fiorillo and Williams 2000) receptors has been demonstrated.

The discrepancy between the effects of A_1 and A_{2A} antagonists resembles differences between D_1 and D_2 dopamine agonists under the same drug discrimination paradigm. In most studies analyzing dopaminergic involvement in the discriminative-stimulus effects of methamphetamine or amphetamine (Munzar and Goldberg 2000; reviewed by Brauer et al. 1997), D_2 agonists produced complete generalization to an amphetamine training stimulus, whereas D_1 agonists produced only partial generalization. Since A_1 receptors are coupled to D_1 receptors (Ferré et al. 1994, 1999; Ginés et al. 2000), the failure of DPCPX to generalize to the methamphetamine-training stimulus is not surprising.

Also, the partial generalization produced by DMPX to the methamphetamine-training stimulus might be explained by an antagonistic interaction of A_{2A} and D_2 receptors (Ferré et al. 1991, 1997), suggesting that DMPX, as an A_{2A} antagonist, removed negative adenosinergic tonus from D_2 receptors and thus mimicked the effects of methamphetamine. This hypothesis is further supported by the ability of the D_2 antagonist spiperone to block the effects of DMPX. The dose of spiperone used also blocked the discriminative-stimulus effects of methamphetamine itself, but to substantially smaller extent, which is in line with a stronger D_2 component in the actions of DMPX. It has to be noted, however, that in the DMPX-spiperone combination tests only rats in which DMPX at least partially generalized to the methamphetamine-training stimulus during initial experiments were used. It is possible that in this selected subgroup of rats the D_2-like effects of methamphetamine were more pronounced.

The generalization produced by DMPX to the methamphetamine-training stimulus was almost completely blocked by SCH 23390, a D_1 antagonist, although this occurred at a dose, which disrupted the animals' behavior.

This finding was unexpected and might be explained by two possible factors. First, although A_{2A} and D_1 receptors are not colocalized in the same neurons, it has been shown that blockade of A_{2A} receptors potentiates the effects of D_1 agonists and counteracts the effects of D_1 antagonists (Pinna et al. 1996; Hauber et al. 1998). These A_{2A}-D_1 interactions can be explained by an interaction at the circuit level (Ferré et al. 1997). Second, even though DMPX appears to reverse selectively the effects of A_{2A} versus A_1 receptor stimulation in vivo (Seale et al. 1988) and has been widely used as an A_{2A} antagonist in pharmacological studies, its in vitro selectivity is rather limited; DMPX exhibits only 3- to 10-fold A_{2A} selectivity depending on the test systems that are being compared (Muller et al. 1997). Thus, the effects of DMPX found in the present study might be related to its ability to block both A_1 and A_{2A} receptors at high doses (Seale et al. 1988). Future studies with more selective compounds appear to be necessary once they become available.

Caffeine partially generalized to the methamphetamine training stimulus in the present study as in previous studies in amphetamine- and cocaine-trained animals (Schechter 1977; Gauvin et al. 1990; Young et al. 1998; reviewed by Garret and Griffiths 1997). However, caffeine unlike DMPX produced generalization to methamphetamine only after doses which markedly decreased rates of responding and was approximately three-times less potent than DMPX, although both compounds are structural analogs of xanthine with only slight differences in their molecular structure (Klotz 2000). These differences between the effects of caffeine and DMPX probably reflect a stronger A_{2A} component of action with DMPX. In contrast to substitution tests, the shift in the methamphetamine dose-response curve produced by caffeine was comparable, if not greater, than the shift produced by DMPX. It is probable that a stronger A_1 component of action with caffeine contributed to this discrepant finding, in line with the observed potentiation of methamphetamine's effects by the A_1 antagonist DPCPX. Simultaneous blockade of both A_1 and A_{2A} receptors by caffeine was likely responsible for the inability of the D_2 antagonist spiperone to reverse its effects, even though spiperone did block effects of the preferential A_{2A} antagonist DMPX. It is also possible that non-adenosinergic components of caffeine's action, such as inhibition of phosphodiesterases (reviewed by Garrett and Griffiths 1997 and by Fredholm et al. 1999), contributed to this observation and to inability of caffeine to produce more pronounced methamphetamine-like responding. In fact, Powell et al. (1999) have recently reported that both D_1 and D_2 receptor antagonists can alter the discriminative stimulus effects of caffeine in animals trained to discriminate a low 10.0 mg/kg dose but not a high 56.0 mg/kg dose of caffeine from its vehicle. This suggests that after higher doses of caffeine non-dopaminergic mechanisms are present as well. These authors also reported that coadministration of caffeine with D_1 antagonists produced a significant disruption in lever pressing (Powell et al. 1999). This resembles the marked reduction in lever pressing observed when SCH 23390 was coadministered with DMPX and caffeine in the present study. The reasons for this interaction are not clear and require future studies.

In conclusion, the present findings confirm the role of adenosine receptors in the discriminative-stimulus effects of methamphetamine. The study further suggests that A_{2A} receptors play a direct role in mediating methamphetamine's discriminative-stimulus effects, whereas A_1 receptors play a rather indirect, albeit important modulatory role. Increasing availability of both antagonists and agonists with more selective actions at different subtypes of adenosinergic receptors than those utilized in the present study (Muller et al. 1997; Klotz 2000) might help to further resolve the mechanisms that underlie adenosinergic involvement in the discriminative-stimulus and other behavioral actions of methamphetamine.

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